Anaerobic Mechanisms in the Diving of the Loggerhead Musk Turtle, *Sternothaerus minor*

By

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INTRODUCTION

Sternothaerus minor is a small, highly aquatic chelydrid turtle confined to the southeastern United States. Tinkle (1958) has provided an account of its distribution and ecology. It is primarily an animal of lotic situations and reaches maximum abundance in the clear, calcareous spring runs of the Suwannee River drainage in north-central Florida. This environment contributed all of the specimens used in my experiments.

The diving ability of turtles has been studied by a number of authors. Gage and Gage (1886) demonstrated the occurrence of aquatic respiration in soft-shelled turtles; Dunson (1960) has repeated and expanded this work. The importance of aquatic respiration in the musk turtle, Sternothaerus odoratus, was investigated by Root (1949). I will discuss this work later. Shaw and Baldwin (1935) stated that "the ability of the turtle to extend a period of apnea over several hours is made possible by several factors, most important of which are very thorough lung ventilation . . . low oxygen consumption . . . a low 'unloading tension' for turtle hemoglobin . . ." As I shall demonstrate below, they were mistaken.

The relationship between body temperature and survival of submergence in the painted turtle, Chrysemys picta, has recently been studied by Musacchia (1959). He found that even at 26°C this species could survive up to 52 hours' forced submergence in aerated water.

The fact that many fresh-water turtles in northern latitudes spend the winter buried in mud under water is well known (Carr, 1952), but little speculation concerning the mechanism of this feat has appeared. Musacchia (1959) advances the view that ". . . the combination of various intrinsic factors, such as decreased oxygen consumption, altered intermediary metabolism and torpidity, and the extrinsic influence of an increased aqueous-oxygen tension in cold water, are acting concomitantly to determine the viability of these turtles." How "altered intermediary metabolism," which consists of ". . . elevated liver glycogenolysis, maintenance of blood sugar level, and low oxygen consumption . . ." (Rapatz and Musacchia, 1957), facilitates their survival is not explained. I shall propose a hypothesis which clarifies this matter in a later section of this report.

The physiological mechanisms responsible for the diving ability of aquatic mammals have received considerable attention. These animals are able to survive long periods of submergence principally by means of their ability to meter stored oxygen to those tissues which would be damaged by anoxia, and to use anaerobic glycolysis elsewhere, developing a large oxygen debt (Prosser et al, 1950). One of the ablest of mammalian divers is the grey seal, Halichoerus grypus, which has been known to remain submerged for 20 minutes (Backhouse, 1954) and could perhaps survive a submergence of half an hour. Most other diving mammals have not been observed to submerge for over 15 minutes, and even the great whales, with their relatively low metabolic rates, cannot survive a submergence of much over two hours (Irving, 1939). When compared with the performances of these famous diving animals, the feats of turtles are impressive. That this ability is not wholly due to the relatively low metabolic rate and great oxygen storage capacity of the turtle will be shown by the following considerations.

Scholander (1940, cited in Krogh, 1941) calculated values for the oxygen storage capacity of a seal, Cystophora, of 29 kg (table 1). Since the average oxygen uptake of this animal at rest is about 550 ml/kg hr, its 1550 ml reserve should last only about five and a half minutes. In actuality, this seal could probably survive a submergence of 30 minutes--five and a half times what one would expect.

Table 2 is a similar inventory of oxygen stores for the musk turtle; the data and assumptions used in its preparation will be found in the appendix. It can be seen that the seal stores relatively more oxygen than the turtle does. At 22°C the oxygen uptake of Sternothaerus at rest varies between about 10 and 30 ml/kg hr (see table 4), so the tabulated oxygen store could be expected to last up to four hours if it could be entirely utilized. If the oxygen-conserving ability of Sternothaerus were equal to that of the seal, it could be expected to survive five and a half times this long, or up to 22 hours.

The times of submergence necessary to drown 20 specimens of Sternothaerus minor in aerated water at 22°C are listed in table 5 (p.22). The mean is 59.65 hours, and the extreme is 168 hours. It is evident that the diving ability of these reptiles is of a different order from that of aquatic mammals.

Such ability suggests that mechanisms different from those of diving mammals may be in use, and I shall show that this is indeed the case. Two such mechanisms are possible. One, aquatic respiration, has

Table 1. Estimated oxygen stores of a 29 kg seal (after Scholander, 1940)

Source	Stored Oxygen (ml)
350 ml alveolar air with 16% O_2	55
4,500 ml blood with 25 vol% O_2	1,125
6,000 g muscle with 4.5 vol% O_2	270
Other tissues and fluids	100
Total	1,550
	53.5 ml/kg

Table 2. Estimated oxygen stores of a 100 g turtle, *Sternothaerus*

Source	Stored Oxygen (ml)
15 ml alveolar air with 18% O_2	2.7
8 ml blood with 10 vol% O_2	0.8
15 g muscle with 2 vol% O_2	0.3
Other tissues and fluids	0.25
Total	4.05
	40.5 ml/kg

been observed and measured in turtles, but has been deemed inadequate to account for their long submergences. The other, survival by means of anaerobic energy production alone, has been suggested by only one study (Johlin and Morland, 1933) and has not been further investigated.

It has been my purpose to make a preliminary assessment of the importance of the latter mechanism.

THE IMPORTANCE OF GLYCOLYSIS IN THE SURVIVAL OF ANOXIC STERNOTHAERUS

A key to the problem of how turtles can endure such long periods of submergence is provided by Johlin and Morland (1933), who found that "fresh water turtles weighing from 1,000 to 1,500 grams each" (lamentably not identified) could survive for 27 hours at 20°C, breathing pure nitrogen. In order to live, any animal requires a metabolic source of energy. Figure 1 is a greatly simplified diagram of the metabolic pathways thought to be ultimately responsible for nearly all energy production in vertebrates (Fruton and Simmonds, 1958). The energy gain resulting from the various steps is represented as adenosine triphosphate (ATP), a common energy-transfer metabolite. The reactions above the dotted line do not require oxygen; those below it do.

Although most of the energy is gained from the aerobic phase, many invertebrates (Von Brand, 1946) and some fishes (Blazka, 1958) are able to survive for long periods using only the energy yield of glycolysis (and perhaps other, unknown anaerobic pathways as well). This ability has not been reported in amniotes, although newborn mammals are better able to withstand anoxia than are adults, and are known to possess relatively better developed glycolytic systems (Cassin and Herron, 1961). I shall demonstrate below that the prolonged survival of anoxic Sternothaerus is due to glycolytic energy production. To do so, I shall compare the survival times of turtles in which only

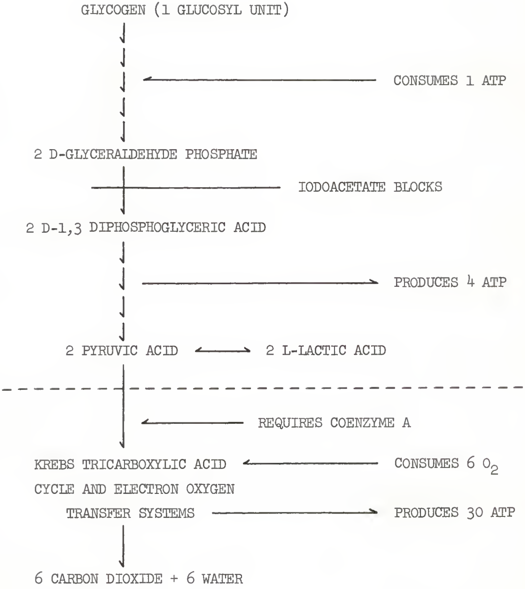

Figure 1. Pathways of energy production in vertebrates

the aerobic phase of energy production is blocked, with those of others in which glycolysis is also prevented.

Materials and Methods

The turtles used in my experiments were *Sternothaerus minor* of both sexes, weighing from 30 to 250 grams. All were collected in the Ichtucknee River, Columbia County, Florida, a clear, calcareous spring-run in which the temperature remains at 22° ± 1°C throughout the year. They were kept in aquaria at 22° ± 4°C and were starved from 7 to 28 days before being used in an experiment.

Turtles were maintained in an anoxic atmosphere by means of the apparatus illustrated in figure 2. At the start of an experiment, a turtle was placed in the chamber which then was completely filled with deoxygenated water through tube A. Helium (Matheson Company, minimum purity 99.99 per cent) was then introduced through tube B, displacing water through tube A until a gas volume of one liter had been attained. The helium source was then connected to tube A, and helium was passed through the chamber at the rate of 200 ml per minute for the remainder of the experiment. The chamber containing the animal was maintained at 22°C by means of a constant temperature water bath. Each experiment was terminated after the turtle had failed to breathe for two hours. Survival time was defined as the period between the first breath of helium and the last.

Oxygen consumption was determined using a volumetric respirometer of my own design, illustrated in figure 3. This apparatus is similar in principle to volumetric respirometers previously used to determine the

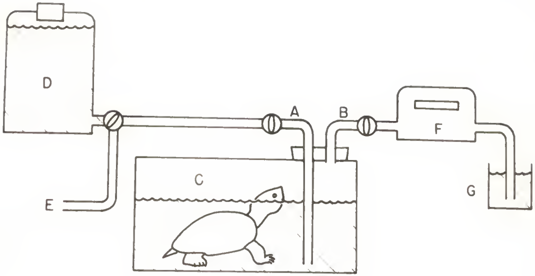

Figure 2. Apparatus used in determining the survival times of anoxic turtles. A, B, see text; C, chamber (dessicator); D, deoxygenated water reservoir; E, helium source; F, Pauling oxygen analyzer (Beckman model C2); G, water valve. Tubing is glass and heavy-walled rubber.

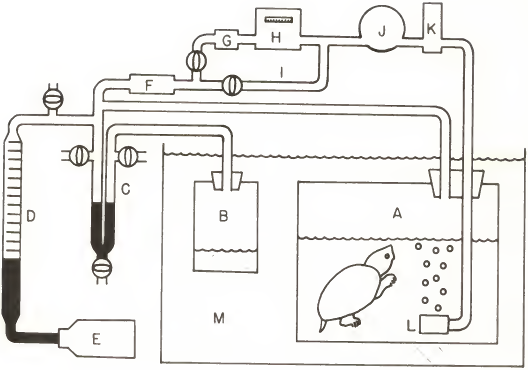

Figure 3. Volumetric respirometer for amphibious animals. A, respirometer chamber (dessicator); B, compensating chamber; C, manometer; D, volumetric burette; E, squeeze bottle (with screw clamp); F, carbon dioxide absorbent (Baralyme); G, dryer (silica gel); H, Pauling oxygen analyzer (Beckman model C2); I, oxygen analyzer by-pass; J, diaphragm pump with controllable flow rate; K, flow meter; L, sintered glass air stone; M, constant temperature water bath. Tubing is glass and heavy-walled rubber.

oxygen uptake of aquatic animals (Scholander, 1949; Calhoon and Angerer, 1953) but differs from them in that the gas phase is circulated, and its oxygen partial pressure is monitored by means of a Pauling oxygen analyzer. Through the equilibrium maintained between the gas and water phases, the total oxygen uptake of organisms using both aquatic and aerial respiration can be measured. The thorough circulation and equilibration of both phases allows this apparatus to be used with larger animals and respirometer chambers than was practicable with other systems of its type. As the animal uses oxygen, it reduces the pressure in the respirometer chamber with respect to that in the compensating chamber. Periodically, the experimenter adds oxygen from the volumetric burette until the pressures are equalized, as shown by the manometer, and records the amount of oxygen required to do this, which is equal to the amount the animal has used since the last measurement. By using the oxygen analyzer and introducing nitrogen or oxygen through the left manometer stopcock before beginning to record oxygen consumption, any desired partial pressure of oxygen from 0 to 760 mm can be maintained.

In order to prevent nearly all anaerobic energy production without greatly interfering with aerobic processes, I injected turtles with iodoacetic acid. This poison has long been known to block glycolysis and has recently been used in studies of anaerobiosis in intact animals (Himwich et al, 1942; Fazekas and Himwich, 1943). It irreversibly inhibits phosphoglyceraldehyde dehydrogenase, blocking its sulfhydryl group by alkylation, and thus blocks glycolysis prior to any energy production (see figure 1). Unfortunately, its specificity is only relative, and in moderate concentrations it also inhibits many other sulfhydryl-containing enzymes, such as coenzyme A. It use in studies such as this one has been attacked on these grounds by Fitzgerald (1956), who expressed the belief that the shortened survival period of iodoacetate-poisoned mammals (as compared to normal ones) in nitrogen atmospheres was due to the animals' inability, because of iodoacetate poisoning of aerobic processes, to use their oxygen stores.

In order to counter this objection, I used a dosage of iodoacetate (15 milligrams per kilogram) which was not lethal to animals given access to air, and which did not interfere greatly with their oxygen utilization. The iodoacetic acid used to poison the turtles was freshly dissolved in distilled water and made up to a concentration of 3 milligrams per ml. It was injected intraperitoneally 24 hours before each animal was used in an experiment, in order to allow its uniform distribution throughout the turtle's tissues.

Table 4 (p.21) compares the oxygen uptake, in air, of iodoacetate-poisoned animals with that of normal ones. The oxygen uptakes of the poisoned animals were measured between 20 and 28 hours after the animals were injected, just before they were subjected to the anaerobic survival experiments. Although the oxygen uptake of the iodoacetate-poisoned animals is somewhat depressed, it falls well within the range of the normal group, showing that the iodoacetate-poisoned turtles were not prevented from using oxygen.

Experiments and Results

The survival times of normal and iodoacetate-poisoned Sternothaerus in anoxic atmospheres (less than 0.01 per cent O_2) were measured by means of the apparatus illustrated in figure 2 and are recorded in table 3. In these experiments I used helium in preference to nitrogen, as the latter appeared to produce narcotic effects. The turtles readily breathed the helium, which was passed through the chamber and exhausted; thus it can be assumed that their tissues, in equilibrium with the gas by means of the lungs and circulatory system, were quickly freed of oxygen.

Discussion

When iodoacetate-poisoned turtles are placed in an anoxic atmosphere, they should have almost no metabolic source of energy, while non-poisoned animals should be able to use glycolysis. A comparison of the survival times of poisoned and normal turtles breathing helium (table 3) gives strong evidence that the means of anaerobic survival of Sternothaerus is indeed glycolysis.

The oxygen consumption of a resting vertebrate in an oxygen-rich environment may be considered a measure of its normal energy requirement. A comparison of the resting oxygen consumption of Sternothaerus with that of a mammal of similar size suggests an explanation of the ability of this turtle to survive for many hours in an anoxic environment in which an adult mammal would die in but a few minutes. Sternothaerus at 22°C uses only about 20 ml oxygen per kilogram hour, while a mammal of the same size (body temperature about 38°C) uses about 1,000, or 50 times as much. This fact, coupled with the great insensitivity of the turtle's brain to anoxia, may help explain the

Table 3. Survival times of *Sternothaerus minor* breathing pure helium (22°C). Each value represents a different individual.

	Survival time (hours)	
	Normal animals	Iodoacetate-poisoned animals
	8.0	0.13
	9.0	0.15
	9.0	0.15
	9.5	0.17
	9.5	0.17
	9.5	0.17
	10.0	0.25
	10.5	0.30
	11.0	0.52
	12.0	0.55
	12.5	0.93
	13.0	
	13.0	
	13.5	
	14.0	
	16.0	
	19.0	
	20.0	
mean	12.2 hours	0.32 hours
standard deviation	3.40 hours	0.25 hours

ability of Sternothaerus to survive in an anoxic environment. The turtle's energy requirement at 22°C is so small that it can be nearly fulfilled by the inefficient mechanism of anaerobic glycolysis alone.

Another factor which may be responsible for the death of anoxic mammals is the accumulation of acid metabolytes, such as lactic acid. Although an anoxic turtle might be expected to derive enough energy for survival from glycolysis so long as its glycogen supply lasted, how would it deal with these toxic byproducts? The following data are instructive. Johlin and Morland (1933) found that the blood glucose level of anoxic turtles rose from a normal value of 50 mg per cent to as high as 1,070 mg per cent. This high concentration probably serves to increase the rate of glycolysis, as can be inferred from the data of Holmes and Holmes (1927), who found that in cats the concentration of cerebral lactic acid developed under anaerobic conditions is proportional to the level of blood sugar. Johlin and Morland (1933) also found that the blood lactate level of anoxic turtles rose to as much as 15 times the normal level, and that the rate of carbon dioxide production was always greater (sometimes by a factor greater than 2) than the rate observed in animals breathing air. The latter fact, strange because most carbon dioxide production in animals normally results from the oxidative processes of the tricarboxylic acid cycle, puzzled these authors. They suggested that the carbon dioxide might come from bone carbonates, since the total tissue oxygen and bicarbonate supplies were insufficient to account for it. Altland and Parker (1955) also noticed that in hypoxic box turtles, Terrepene carolina, oxygen consumption was reduced, but carbon dioxide production was not.

I suggest that, in fact, much of this carbon dioxide is coming from the decarboxylation of some acid metabolyte resulting from glycolysis, and that after decarboxylation this metabolyte is transformed into fats. This hypothesis is supported by the work of Blazka (1958), who found that in anoxic crucian carp, Crassius crassius, lactic acid and other such acid metabolytes are neither accumulated nor excreted. Moreover, fats are accumulated by these fish during anoxia, and the only acid metabolyte excreted is carbon dioxide. The high levels of blood lactate observed in anoxic turtles may represent an equilibrium concentration necessary for this modification of the normal glycolytic pathway and may reflect the fact that, from an evolutionary standpoint, the ability of these turtles to survive anoxia at 22°C is fortuitous, since such conditions are not encountered by them in nature.

The survival of turtles wintering in anaerobic environments such as the bottom mud of ponds may thus be explained in terms of anaerobic energy production. At the temperatures of such situations (4°C and lower), the energy requirement of a turtle would presumably be so small that its glycogen stores could last for several months. An oxygen consumption of 3.17 ml per kilogram hour has been reported for Chrysemys picta at 5°C (Rapatz and Musacchia, 1957). Musacchia (1959) found that at 3.5°C this species survived for an average of 63 days when confined under water in sealed chambers containing no gas phase. These animals probably would have survived still longer if carbon dioxide had been prevented from accumulating in their aquatic environment. It would be interesting to compare the fat and glycogen contents of turtles before and after a long period of anoxic cold torpor.

THE IMPORTANCE OF AQUATIC RESPIRATION TO DIVING STERNOTHAERUS AND THE ROLE OF GLYCOLYSIS IN AQUATIC RESPIRATION

In his admirable book, The Comparative Physiology of Respiratory Mechanisms, Krogh (1941) stated, "The amphibia can remain very long under water, thanks to their cutaneous respiration, but in all others the uptake of oxygen through the skin is insignificant." In this he was mistaken. Sternothaerus minor is able to make good use of oxygen dissolved in the water in which it is diving. The aquatic respiration of the closely related species Sternothaerus odoratus was studied by Root (1949). He determined the extent of the aquatic oxygen uptake and, by selectively masking the heads and bodies of his turtles, found that about two thirds of the oxygen was absorbed cutaneously and that the remaining third was absorbed buccopharyngeally. In his measurements of aquatic oxygen consumption, Root sealed each turtle in a vessel containing a known volume of water with a known oxygen content. After about four hours he sampled this water for dissolved oxygen, using the Winkler technique, and was able to calculate the oxygen consumption of the turtle.

I have measured the oxygen consumption of both air-breathing and forcibly submerged Sternothaerus minor, using the volumetric respirometer previously described, in which circulation of the gas phase permits a constant partial pressure of oxygen to be maintained. This system avoids one objection to Root's technique, namely, that the concentration of dissolved oxygen available to his submerged turtles decreased throughout each experiment.

In order further to assess the contribution of aquatic respiration to the survival of diving Sternothaerus, I compared the survival times of individuals confined beneath aerated water with those of others confined beneath anoxic water.

Finally, I attempted to determine the relationship between the capability of Sternothaerus to endure anoxia and its ability to utilize oxygen dissolved in water. This I did by comparing the survival times of normal and iodoacetate-poisoned turtles confined under aerated water, and by comparing the abilities of normal and iodoacetate-poisoned turtles to utilize oxygen at low partial pressures.

Materials and Methods

The turtles used and the iodoacetate-poisoning procedures were the same as those in the previous section. Aquatic oxygen consumption was measured by means of the respirometer described previously (figure 3, p. 10), modified only in that the turtle in the respirometer chamber was confined in a wire cage so that it could not reach the gas phase.

In order to determine their survival times, I submerged turtles in a 20-liter aquarium, fitted with a gas-tight lid and nearly filled with water, so that during an experiment it contained about one liter of gas. Air or helium (Matheson Company, minimum purity 99.99 per cent) was introduced through a sintered glass bubbler at the bottom of the aquarium and exhausted through a small-diameter tube at the top. It was passed through the system at the rate of 250 ml per minute and served to circulate the water vigorously. The turtles were contained in a wire cage which prevented them from reaching the surface or the bubbling gas. The temperature of the aquarium and its contents was maintained at $22^{\circ} \pm 1^{\circ}C$. At the beginning of each experiment, five turtles were placed in the wire cage, which was partly lowered into the aquarium so that the turtles were still able to come to the surface and breathe. After each turtle had been observed to ventilate its lungs thoroughly at least once, the cage was completely submerged, gas bubbling was begun, and, if helium was being used, the system was sealed. When helium was used, the partial pressure of oxygen in the gas leaving the system was found to drop to less than 5 mm Hg within the first ten minutes of bubbling. A turtle was deemed dead when a glass rod touched to its cornea first failed to elicit the eye-blink reflex. About one in ten turtles in this condition could be revived.

In the sealed chamber experiments, the apparatus illustrated in figure 2 was used. After a turtle was sealed in the chamber with enough water to give a gas volume of about 600 ml, helium or nitrogen was passed through the system, entering through tube A and exhausting through tube B, until the partial pressure of oxygen in the chamber was reduced to 30 mm ± 10 mm Hg. Tubes A and B were both then filled with water, both stopcocks were closed, and the chamber containing the turtle was placed in a constant temperature water bath at $22^{\circ} \pm 1^{\circ}C$. After the turtle appeared dead, the gas in the chamber was displaced through tube B to the oxygen analyzer by introducing deoxygenated water through tube A.

Experiments and Results

The aquatic oxygen uptake of Sternothaerus minor confined under aerated water (partial pressure of oxygen 152 mm Hg) is compared with that of individuals breathing air in table 4, where these data should be compared with those of Root (1949) for Sternothaerus odoratus at 25°C. The much greater average oxygen uptake in air of Root's turtles as compared to mine may partly be attributed to the higher temperature at which his measurements were made, but undoubtedly a more important factor was the greater average activity of his turtles. I left each of my turtles in the respirometer chamber for at least 24 hours before measuring its aerial oxygen uptake, with the result that a much larger proportion of them were inactive.

In table 5, the survival times of Sternothaerus minor confined under water equilibrated with helium (and thus anoxic) can be compared with those of individuals confined under aerated water. It is obvious that aquatic respiration is of considerable importance.

It is noteworthy that the rhythmic throat movements which circulate water through the buccopharyngeal area were augmented in turtles confined under aerated water and were greatly reduced in turtles confined under water in equilibrium with helium. This suggests that, contrary to opinions expressed in the literature (McCutcheon, 1943; Root, 1949), the respiratory function of these movements is not entirely fortuitous and, further, that these turtles may have some means of sensing the oxygen content of their aquatic environment.

The survival times of iodoacetate-poisoned turtles confined under aerated water (table 5) are shortened to about what one would

Table 4. Oxygen consumption of Sternothaerus. Each value represents the mean oxygen uptake during 5 hours of a different turtle.

Oxygen uptake, ml per kg hr reduced to 0°C and 760 mm Hg

Iodoacetate-poisoned S. minor at 22°C breathing air	Normal S. minor at 22°C breathing air	Normal S. minor at 22°C confined under aerated water
37.1	95.0	9.55
27.6	82.0	8.50
25.3	60.1	8.35
25.0	56.2	8.05
22.3	53.8	7.90 **
22.3	43.1	7.80
20.7	41.6	7.75 *
20.7	40.5	7.50
20.3	38.1	7.25
19.2	36.5	6.90 **
	35.5	
	33.2	
	33.0	
	32.7	
	32.3	
	31.9	
	31.4	
	31.1	
	31.0	
	30.8	
	30.5	
	30.4	
	29.7	
	28.8	
	27.0	
	26.0	
	24.0	
	23.2	
	22.5	
	21.3	
	13.2	
	11.0	
	10.5	
	10.3	
	9.9	
	9.5	
mean 24.05 ml/kg hr	mean 33.3 ml/kg hr	mean 7.96 ml/kg hr
standard deviation 5.29 ml/kg hr	standard deviation 18.3 ml/kg hr	standard deviation 0.74 ml/kg hr

* mean for 2 turtles
** mean for 5 turtles

Normal S. odoratus at 25°C breathing air (from Root, 1949)

35 observations
range 19.1 - 129.2

mean 70.6 ml/kg hr

standard deviation 25.8 ml/kg hr

Normal S. odoratus at 25°C confined under aerated water (from Root, 1949)

25 observations
range 4.64 - 12.72

mean 8.70 ml/kg hr

standard deviation 1.82 ml/kg hr

Table 5. Survival times of *Sternothaerus minor* confined under water at 22°C. Each value represents a different individual.

Survival time (hours)		
Normal turtles in water equilibrated with pure helium	Normal turtles in aerated water	Iodoacetate-poisoned turtles in aerated water
8.0	27	1.25
9.0	29	1.50
9.0	31	1.75
9.0	36	1.75
9.5	38	2.00
9.5	41	2.00
9.5	44	2.25
10.0	47	2.25
10.0	49	2.25
10.5	50	2.50
10.5	51	2.50
11.0	53	2.50
11.5	58	2.50
12.5	62	2.75
13.5	63	2.75
14.0	67	2.75
16.0	74	3.00
18.0	86	3.00
21.0	119	3.00
22.0	168	3.25
23.0		
mean 12.7 hr	mean 59.7 hr	mean 2.38 hr
standard deviation 4.61 hr	standard deviation 33.4 hr	standard deviation 0.57 hr

expect if only oxygen stores were used. Evidently iodoacetate poisoning greatly curtails the turtles' ability to survive submergence. Since I have shown that a turtle's capacity for oxidative metabolism is not greatly affected by the dosage of iodoacetate used (see table 4), this phenomenon is probably due mainly to the failure of glycolysis, rather than to a direct disfunction of any oxidative mechanism. The following experiments help to clarify this.

Normal and iodoacetate-poisoned Sternothaerus minor were placed in sealed chambers with gas mixtures containing insufficient oxygen to supply the animals' minimum normal rate of oxygen consumption for more than 10 to 20 hours. After the turtles appeared dead, the gas in the chambers was analyzed to determine how low the turtles could bring the partial pressure of oxygen in their environment before they succumbed. The results of these analyses are presented in table 6. They indicate that even iodoacetate-poisoned turtles should be able to use at least 80 per cent of their stored oxygen when confined under water.

Discussion

In his study of the aquatic respiration of Sternothaerus odoratus, Root (1949) concluded that since the aquatic oxygen uptake of his turtles, on the average, was equal to only about one eighth of their average oxygen uptake in air, aquatic respiration did not contribute significantly to the ability of these animals to remain submerged. My comparison of the survival times of turtles confined under aerated and anoxic water (table 5) indicates that this conclusion was incorrect.

In well-aerated water the usefulness of aquatic respiration to

Table 6. Oxygen partial pressures associated with the death of Sternothaerus minor in sealed chambers at 22°C. Each value represents a different individual. Accuracy of these measurements is ± 1.6 mm Hg.

Partial pressure of oxygen in mm Hg			
Normal animals		Iodoacetate-poisoned animals	
initial	final	initial	final
38.0	1.0 *	40.0	6.0
29.0	1.0 *	32.0	11.5
25.5	1.0	32.0	12.0
22.0	1.0 *	32.0	13.0
20.5	1.0	30.0	13.0
20.0	1.0	32.0	14.0
18.0	1.0	32.0	15.0
18.0	1.0	45.0	16.0
17.0	1.0 *	43.0	18.0
16.0	1.0 *	32.0	18.0
15.5	1.0	32.0	19.0
155.0	1.5	34.0	19.0
25.5	1.5	22.0	19.5
24.0	1.5	30.0	20.0
23.0	1.5	35.0	20.0
22.0	1.5	35.0	20.5
21.0	1.5	32.0	21.5
17.0	1.5	35.0	23.0
34.5	2.0		
21.5	2.0		
19.5	2.0	mean	16.6 mm
		standard deviation	4.35 mm
mean	1.3 mm		
standard deviation	0.37 mm		

* animal recovered

turtles would presumably be inversely related to temperature. As the temperature was lowered, the oxygen content of the water would increase, while the oxygen requirement of the turtles would decrease. On this basis, it might be possible to explain the survival of turtles wintering under aerated water in terms of aquatic respiration alone.

In demonstrating that the low partial pressure of oxygen was the factor responsible for the death of animals at high altitude, Bert (1878) placed various animals in sealed chambers, waited until they died, and analyzed the atmospheres remaining in the chambers. Although he varied the initial composition of the atmosphere, the temperature, the total pressure, and other factors, he found that for each species there was a characteristic narrow range of oxygen partial pressures below which the animals could not survive. Some of his data are presented in table 7. Compare these values with those which I obtained from Sternothaerus minor (table 6). The iodoacetate-poisoned turtles, dependent entirely on aerobic respiration, yield values similar to those obtained by Bert from birds and mammals. The normal turtles are able to extract practically all the oxygen from their gaseous environment.

I suggest that this ability is the result of the capacity of Sternothaerus to survive for a relatively long time using the energy yield of glycolysis alone. Thus its tissue oxygen partial pressures can reach levels so low that the resulting gradient permits the uptake of oxygen from the environment, despite the low oxygen partial pressure of the latter. This hypothesis also provides an explanation of the difference between the survival times of normal and iodoacetate-

Table 7. Oxygen partial pressures associated with the death of animals in sealed chambers (after Bert, 1878)

Species	Partial pressure of oxygen (mm Hg)
house sparrow (*Fringilla domestica*)	22.8 - 32.7
mountain sparrow (*Fringilla montana*)	24.3 - 34.2
owl (*Strix psilodactyla*)	25.1 - 38.0
kestrel (*Falco tinnunculus*)	38.8
cat (adult)	30.4 - 37.2
cat (three days old)	16.7 - 18.2
dog	22.8 - 28.1
rabbit	24.3 - 31.9
rat (adult)	average 15.2
rat (young)	average 5.7
guinea pig	15.2 - 25.8
frog	16.7 - 21.3
poplar beetle (*Chrysomelida*)	no oxygen detectable

poisoned animals confined beneath aerated water (table 5). Evidently the oxidative processes in the iodoacetate-poisoned turtles are alone unable to provide sufficient energy to allow the animals to survive with very low tissue oxygen partial pressures, and thus these animals are not only denied the energy yield of glycolysis, but also prevented from utilizing oxygen dissolved in the water.

SUMMARY AND CONCLUSIONS

The basis of the ability of Sternothaerus minor to endure long periods of submergence was investigated by comparing the survival times of animals subjected to various interferences with energy production, and by measuring their oxygen consumption.

Normal turtles are able to endure anoxia for an average of 12.2 hours at 22°C. Turtles whose glycolytic energy production is almost completely prevented by iodoacetate poisoning can endure anoxia for an average of only 0.32 hours at this temperature. It is concluded that the basis of survival of anoxic turtles is the energy yield of anaerobic glycolysis.

At 22°C the mean oxygen uptake of turtles confined under aerated water is about one fourth that of individuals breathing air. At this temperature, turtles confined under aerated water survive for an average of 59.7 hours, while others confined under anoxic water survive for an average of only 12.7 hours. It is concluded that aquatic respiration is important to this species in enduring long periods of submergence. Further experimental results suggest that the ability of Sternothaerus to utilize oxygen dissolved in water is dependent upon its capacity to endure extreme tissue hypoxia.

Under the conditions of cold torpor experienced by wintering turtles, it is likely that either aquatic oxygen uptake or anaerobic glycolysis could account for an under-water survival of several months' duration.

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APPENDIX

Sources of Stored Oxygen in Sternothaerus, with Data and Assumptions Resulting in Estimates of Their Oxygen Content at the Start of a Dive

Alveolar air

Volume. Field and laboratory observations have indicated that a diving Sternothaerus almost always carries just sufficient air in its lungs to give it neutral buoyancy at a depth of about six inches. This makes possible the following method for estimating lung volume at the beginning of a dive. The trachea of a freshly killed turtle is cut and a tight-fitting cannula is inserted into the cut end leading to the lungs. The cannula is attached to a volumetric syringe with which air can be introduced into the turtle's lungs. The latter are then deflated completely, and the turtle is placed six inches deep in a water tank. Air is then delivered to the lungs until neutral buoyancy is obtained, the volume necessary to do this being measured. Sixteen such measurements of Sternothaerus minor resulted in a range of from 10.7 to 15.2 ml per 100 g turtle weight, with a mean of 13.2 ml per 100 g. It can be seen that a 100 g specimen would not take much more than 15 ml.

Oxygen content. Wilson (1939) found that the partial pressure of oxygen in the alveolar air of Pseudemys troosti (= scripta) when the turtle was resting out of water varied from 86 mm to 126 mm Hg.

Similar measurements of Clemmys rivulata by Basoglu (1950) produced values of from 122 mm to 137 mm Hg. Since Sternothaerus normally takes from 5 to 20 rapid deep breaths before diving (reminding one of the voluntary hyperventilation of some human swimmers), I shall assume that its lung ventilation is sufficiently thorough to produce an initial average intrapulmonary oxygen partial pressure of 137 mm Hg, or 18 per cent oxygen.

Blood

Volume. The blood weight of a turtle can be expected to be between 6 and 10 per cent of the body weight, as it is in nearly all tetrapods (Prosser et al, 1950, p. 538). Derrickson and Amberson (1934) found a value of 7.56 per cent for Chrysemys picta, which is about equivalent to Sternothaerus in its ability to endure forced submergences (Musacchia, 1959). I shall assume that a 100 g Sternothaerus contains 8 g (= 8 ml) of blood.

Oxygen content. Turtle blood generally has a lower oxygen capacity than mammal blood. Volume per cent values reported for turtles are a maximum of 9.12 for Pseudemys troosti (= scripta) (Wilson, 1939) and 10.85 for P. concinna (Southworth and Redfield, 1926). Since even at the start of a dive all of the turtle's blood cannot be expected to be saturated with oxygen, my estimate of 10 volume per cent is probably somewhat high.

Muscle

Dissection and weighing indicate that the muscles of a Sternothaerus constitute about 15 per cent of its total weight. The muscles are light colored (lighter than beefsteak) and probably contain little myoglobin. My estimate of two volume per cent oxygen content is likely to be a bit above the actual value.

Other tissues and fluids

Not including blood and muscle, a 100 g Sternothaerus would probably contain about 50 ml of water and other fluids, in which oxygen could be dissolved in simple physical solution. Fifty ml of water in equilibrium with oxygen at a partial pressure of 137 mm Hg at 22°C would contain 0.27 ml of dissolved oxygen. Since the mean intracellular partial pressure of oxygen is certainly less than 137 mm, my estimate of 0.25 is surely too large.

BIOGRAPHICAL SKETCH

Daniel Arthur Belkin was born at Los Angeles, California, on July 23, 1934.

He attended the University of California at Los Angeles, where he was influenced by professors G. A. Bartholomew and R. B. Cowles, among others, and was graduated with the degree of Bachelor of Arts in 1956. He next attended the University of Florida, where he held two graduate assistantships, a teaching assistantship, and two National Science Foundation Cooperative Graduate Fellowships.

He is a member of Phi Beta Kappa, the Cooper Ornithological Society, and the American Society of Ichthyologists and Herpetologists.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 5, 1961

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